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EDITORIAL

Is hepatitis C subtyping still relevant in the era of direct-acting antiviral therapy?

Mohammed Eslam · Jacob George

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Hepatitis C virus (HCV) infects ~3 % of the world population and an estimated 3–4 million new infections occur each year [1]. Not only is the virus a leading cause of chronic liver disease, but it is also associated with extrahepatic complications including type 2 diabetes and cardiovascular disease [2, 3].

The HCV is a member of the Flaviviridae family of RNA viruses and is classified into seven recognized genotypes (1–7) [4]; these are further classified into 67 confirmed and 20 provisional subtypes [4]. Each genotype differs from the others by 30–35 % in its nucleotide sequence, while subtypes within each genotype differ by 20–25 % [4]. This remarkable genetic diversity creates a challenge for the development of both HCV vaccines and pan-genotypic drug therapies [4].

Apart from nucleotide sequence variability, HCV genotypes differ in their geographic distribution, rates of viral clearance, risk of progression of liver fibrosis, predilection to hepatic steatosis, the risk of development of hepatocellular carcinoma and in the response to therapy [4–10]. HCV genotype 1 is the most prevalent genotype and the major subtypes, 1a and 1b, are common in North America, Europe and Australia. Genotype 1a is more common than 1b in the United States, while the converse is true in Western Europe [5]. Genotype 2 is dominant in West Africa and south Asia and genotype 3 in south Asia, parts of Europe, the United States and Australia. Genotype 4 frequencies are highest from central Africa to the Middle East, particularly in Egypt, while genotype 5 has higher frequencies in southern Africa and genotype 6 is common in East and Southeast Asia.

As regards the difference in other clinical characteristics, we recently reported that HCV genotype 1 is associated with greater spontaneous clearance compared to genotype non-1 [8]. HCV genotype 3 has also been shown to be associated with virus-induced hepatic steatosis and accelerated fibrosis progression compared to the other HCV genotypes [6, 10]. HCV genotype 1b has been suggested to be a risk factor for hepatocellular carcinoma development, a finding supported by a recent meta-analysis [7].

In spite of the quantum improvements in HCV therapies, the duration and regimen for treatment, types of resistance mutations, cure rates and the need for adjuvant interferon and ribavirin with the new direct antiviral drug (DAA) therapies will remain dependent in part on HCV genotype and subtype, as well as on the cost of treatment in less privileged parts of the world. Thus, more detailed knowledge of the epidemiology and characteristics of HCV genotypes and subtypes is invaluable from a clinical and research perspective and for helping in the development of national treatment policies using the new and old agents.

In this issue of Hepatology International, Andriulli et al. [11] present the results of a large Italian multi-center cohort study ($n = 1{,}233$) of the differences between HCV 1a and 1b subtypes in response to pegylated interferon and ribavirin combination therapy, including a meta-analysis of studies of treatment outcome between patients with HCV subtype 1a and 1b. As expected from the known geographic distribution of HCV subtypes, the majority of subjects in this Italian cohort were infected with HCV 1b (87 %). Subjects with HCV 1a were more likely to be

M. Eslam · J. George
Storr Liver Unit, Westmead Millennium Institute, Westmead Hospital and University of Sydney, Sydney, NSW, Australia

J. George (✉)
Department of Medicine, Westmead Hospital, Westmead, NSW 2145, Australia
e-mail: jacob.george@sydney.edu.au

younger, male, and to have less cirrhosis and type 2 diabetes prevalence. No difference in *IFNL3* (formerly known as *IL28B*) genotype distribution and HCV RNA-levels were observed between groups.

An interesting practice point for clinicians from this study is the influence of statistical power to modify the results. In the present report of 159 patients infected with HCV 1a and 1,074 infected with HCV 1b, the 8 % higher cure rates in the former was not significant. However, if the cohorts sizes were more similar (e.g., 600 per group), this difference would have been highly significant ($p = 0.009$). In this instance, a retrospective power analysis is recommended to ascertain the power of the test after a study has been conducted and non-significant results obtained, to guard against wrongly declaring the null hypothesis to be true and to ascertain that the negative results are not owing to low statistical power [12, 13]. Along the same vein is the differential weight of the predictive factors between the two HCV subtypes. While in subjects infected with HCV 1a, *IFNL3* was the best predictor exceeding RVR, in those infected with 1b, the converse was true. Similarly, the differential role of other predictors such as female gender, age, GGT and viral load from this study (and the lack of importance of liver fibrosis) needs to be interpreted with caution given the markedly asymmetric cohort sizes. This is highlighted by the results of their meta-analysis of 8 previous studies ($n = 4{,}467$) that failed to confirm an association of HCV 1 subtype and cure rates (OR 0.98, CI 0.72–1.32). Surprisingly, liver fibrosis was not a predictor in both subtypes. Interestingly, a robust reverse association with biological plausibility has been observed with the new HCV-targeted protease inhibitors (PIs), with subtype 1a appearing more resistance-prone and less responsive to triple therapy than subtype 1b [14]. A similar observation has been noticed with genotype 3, which appears to be less susceptible to the first generation of DAA PIs, telaprevir and boceprevir, and also to polymerase inhibitors including sofosbuvir [15].

Even with the new DAA therapies, the available data to date suggests that these high efficacious pan-genotypic combinations will continue to require stratification by viral genotype and subtypes to define the optimal duration of drug therapy and to avoid the emergence of drug-resistant mutations [16]. For example, the first generation PIs, telaprevir and boceprevir, have sensitivity only for HCV genotype 1. With approval of sofosbuvir as the first pan-genotypic agent, the requirement for pegylated interferon will still be dependent on HCV genotype. Thus, for HCV genotype 1, high SVR rates (89–90 %) have been reported after treatment with only 12 weeks of sofosbuvir in combination with pegylated interferon and weight-based ribavirin in treatment-naive patients, with no extra value from extending therapy to 24 weeks. On the other hand, SVR rates declined to 68–84 % with sofosbuvir plus weight-based ribavirin in the absence of interferon, after 12 or 24 weeks [16]. For genotypes 2 and 3, sofosbuvir together with weight-based ribavirin for 12 weeks has been approved with SVR up to 97 %, though genotype 3-infected patients may benefit from prolongation of therapy to 24 weeks [16].

The resistance profile of the new DAA's also exhibits differences according to HCV subtype. Telaprevir and boceprevir have a relatively low genetic barrier to resistance, with single nucleotide substitutions leading to high-level resistance [17]. Virologic failure with telaprevir in genotype 1a patients is mainly associated with the variants V36M and R155K alone or in combination; these variants are generally not found in 1b. In the latter V36A, T54A/S, and A156S/T/V appear to be the mutants most conferring resistance [18]. Similarly, with boceprevir, the main resistant variants in 1a are V36M, T54S and R155K, while in 1b the common mutations are T54A/S, V55A, A156S, and I/V170A. However, the median time for all resistance-associated amino acid variants (RAVs) to become undetectable by population sequencing is not significantly different between genotype 1a and 1b [1.11 years (range 1.05–1.2)] [18].

Even with the second wave of HCV-antiviral drugs approved in 2014 (Table 1) (sofosbuvir, simeprevir, Ledipasvir), resistance as a clinical concern is not obviated and particularly for simeprevir, resistant variants appear to vary according to HCV genotype 1 subtype. The resistant variants for most second-wave PIs are R155K in genotype 1a and substitutions at position 168 (D168A/V/E/T) in both 1a and 1b [19]. Clinical data also suggest that the response to simeprevir plus pegylated interferon and ribavirin is reduced in patients infected with HCV genotype 1a and a baseline Q80K substitution [19], the prevalence of which demonstrates marked geographic variation.

Even with new interferon-free all-oral regimens, HCV genotyping is likely to remain valuable for the foreseeable future, at least for certain subgroups. For example, unlike ledipasvir which is only active against genotype 1, one of the promising drugs in the next generation of NS5A inhibitors (GS-5816) exhibits pan-genotypic efficacy. Previous results of a phase 2 trial of sofosbuvir plus GS-5816 for 12 weeks with or without RBV for treatment naïve patients with genotypes 1–6 suggested SVR rates of 91–100 % with 12 weeks of therapy. Based on these results, preliminary data of a trial to evaluate the efficacy of an abbreviated regimen (8 weeks) have been released revealing SVR results of 77–90 %. Genotype 1 patients demonstrated responses rate of 81–90 %, with genotype 2 having rates of 77–88 %. Notably, a quarter of genotype 1 patients and half of genotype 2 patients had NS5A resistance-associated viral variants prior to treatment. Genotype

Table 1 The current approved therapies for chronic hepatitis C in 2014

Drug	Date of approval	Indication	Usage
Sofosbuvir	FDA approved December 6, 2013	HCV Monoinfection HCV-HIV Coinfection	GT 1,4,5,6: Sofosbuvir + peginterferon + ribavirin (12 weeks)
			GT 2: Sofosbuvir + ribavirin (12 weeks)
			GT 3: Sofosbuvir + ribavirin (24 weeks)
Simeprevir	FDA approved December 6, 2013	HCV Monoinfection	GT 1: Simeprevir(12 weeks) + peginterferon + ribavirin (12 or 36 weeks)
			G1 IFN-ineligible patients: Sofosbuvir + simeprevir ± ribavirin (12 weeks)
Ledipasvir plus Sofosbuvir	FDA approved October 10, 2014	Chronic HCV genotype 1	GT 1: Naïve with or without cirrhosis (12 weeks)
			GT 1: Treatment experienced without cirrhosis (12 weeks)
			GT 1: Treatment experienced with cirrhosis (24 weeks)

1 patients had similar cure rates regardless of the presence or absence of resistance variants (86 vs 88 %, respectively), but among people with genotype 2, those with baseline resistance variants had a lower response rate (81 vs 94 %) [20].

For treatment of the experienced patient with genotype 1 or 3, the SVR12 rates in genotype 3 patients with and without cirrhosis were 88 and 100 %, respectively, while the SVR12 rate was 100 % in genotype 1. Among genotype 3 patients, SVR12 rates were higher with the 100 mg compared with the 25 mg GS-5816 dose, and with adding ribavirin [21].

In total, these data suggest that HCV genotyping and subtyping will remain relevant for the foreseeable future as long as genotype-specific differential response rates persist, a scenario most likely in difficult-to-treat subgroups such as those with cirrhosis and baseline resistant mutations.

In conclusion, the Holy Grail in HCV therapies will be the development of "one size fits all" regimens where classical host and viral predictors of response to therapy will cease to be relevant to therapy. Till that day arrives, HCV genotyping and subtyping will remain part of the clinical armamentarium for best clinical practice.

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